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Pharmacophore-based virtual screening: The discovery of novel methionyl-tRNA synthetase inhibitors

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Abstract—We have performed virtual screening of a chemical database of 508,143 commercially available chemicals to search for new methionyl-tRNA synthetase (MetRS) inhibitors. In this study, potent lead compounds with a novel skeleton, including compound **27** with IC_{50} = 237 nM, were successfully identified as *Escherichia coli* MetRS inhibitors.

An aminoacyl-tRNA synthetase (aaRS) is an enzyme that catalyzes the binding of a specific amino acid to its corresponding tRNA to form an aminoacyl-tRNA, which is a substrate for translation in protein synthesis, and is pivotal in determining how the genetic code is interpreted as amino acids.[1] Although the activities of aminoacyl-tRNA synthetases are essential in all living organisms, the selective inhibition of pathogen synthetases over their human cellular counterparts provides an attractive antibacterial mode of action for discovering novel classes of antibiotics, particularly for the treatment of antibiotic-resistant bacterial strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE).[2–4]

The aminoacylation reaction is a two-step mechanism. In the first step, the synthetase recognizes the appropriate amino acid and activates it with ATP to form the activated intermediate, an aminoacyl-adenylate (aa-AMP). The second step transfers the aminoacyl moiety onto the 3′-hydroxyl of the CCA end of the tRNA. The aminoacyl adenylate has been exploited as a starting prototype in the search for novel aaRS inhibitors because of its tight-binding affinity, which is generally two or three orders of magnitude greater than those of amino acid and ATP substrates.

Over the past few years, there have been comprehensive attempts to search for compounds that can specifically target bacterial aaRSs and resultingly inhibit bacterial growth. Modifications of aminoacyl adenylates have been extensively investigated for the purpose of improving their physico-chemical stability, binding affinity, and pathogen selectivity. Many of the modifications have focused on three pharmacophoric regions, including the adenine base, ribose ring, and acylphosphate moieties, to discover stable and more potent surrogates of aminoacyl adenylates as novel antibiotic candidates.[5–16]

Virtual screening of chemical databases is a fast emerging technique and an effective alternative to high-throughput screening (HTS) in drug discovery. It mainly consists of the handling and screening of large chemical databases, in order to reduce the number of chemicals for which the prediction of a specific biological activity has been previously made, using clustering and similarity searching.

As our continuing effort to discover the leads of aaRS inhibitors, we have performed virtual screening of a

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chemical database of 508,143 commercially available compound collections (ChemDiv Inc.) to find the scaffolds of MetRS inhibitors based on the principal pharmacophores of methionyl adenylate. The pharmacophore query used in the 2D-database search was constructed as shown in Figure 1. It was postulated that the A-, B-region, and R^2 fragment of the query would occupy the binding sites of MetRS by interacting with the region comprising the adenine base to the ribose ring, the acylphosphate moiety, and the amino acid side chain of the methionyl adenylate, respectively. In the query, the substituent R^1 of the phenyl group has hydrogen-bonding characteristics similar to those of carboxylic acid, amide, sulfonamide, and nitric oxide, and may act as the amine or ring-nitrogen of adenine group. The lipophilic R^2 is an optionally substituted aryl or heteroaryl ring that is supposed to mimic the lipophilic side chain of methionine. X^1 and X^2 are linkers connecting each pharmacophore.

The amide in B-region is a hydrophilic functional group and may be a good surrogate for the acylphosphate moiety of methionyladenylate.

A structure search was executed using the ChemoSoft program (ChemDiv Inc.) and controlled by matching minimal and maximal percentages of matching (100%: full match, 0%: no matching). To retrieve similar compounds from the 2D database, the minimal percentage was set at approximately 95%. Virtual screening of the ChemDiv diversity set with the query revealed 91 potential candidates with novel scaffolds as MetRS inhibitors. The chemical structures are shown in Table 1. The compounds were evaluated as inhibitors of *Escherichia coli* MetRS, by measuring the decrease of the aminoacylation product, the [^{35}S]methionyl $tRNA^{Met}$ of *E. coli*, in the presence of different chemical concentrations, using scintillation proximity assay (SPA) technology with some modifications.[17,18]

The enzyme inhibitory activities of the tested compounds ranged between nanomolar and micromolar inhibition against *E. coli* MetRS as summarized in Table 2. The most potent compound (**27**) showed an IC_{50} value of 237 nM against *E. coli* MetRS. The pharmacophoric analysis indicated that the 4-benzoic acid in **27** probably can function as the adenine base of the methionyl adenylate. The carbonyl oxygen (C=O) and hydroxyl group (OH) of benzoic acid appear to correspond to the N1 atom (C6=N1) and N6 amine group (NH_2) of the adenine base, respectively. The hydrophilic B-region (X^1-amide-X^2) was proposed to be a ribose-acylphosphate surrogate, because of its hydrogen-bonding characteristics. Since the terminal R^2 part (thiophene) resembles a methionine side chain, in terms of its physico-chemical properties and shape, the moiety may act as an appropriate bioisostere for the ethylmethylsulfide in methionyl adenylate. Compound **24** (IC_{50} = 22.8 μM), where the thiophene ring is replaced by a 3-bromobenzene, displayed a dramatic decrease in the enzyme inhibition, ca. 100-fold, as compared to that of compound **27**. This result indicates that the sulfur atom plays an important role and may interact directly with MetRS. Recently, the 3D structure of MetRS from *E. coli* complexed with methionyl adenylate (Met-$NHSO_2$-AMP) was determined by X-ray crystallography.[19] The methionine-binding pocket was reportedly composed of residues Ala12, Leu13, Tyr15, Trp253, Ala256, Pro257, Tyr260, Ile297, His301, and Trp305. The sulfur atom of the methionine interacts with the phenolic hydroxyl of Tyr260 and the protonated *N*-epsilon atom of His301 by hydrogen bonding. Compound **4**, containing 2-benzoic acid (A-region), $NHCOCH_2CONH$ (B-region), and 6-bromo-1,3-benzothiazole (R^2), exhibited similar inhibition potency as compound **24**. The position of carboxylic acid at the aryl moiety may not be critical for the interaction with MetRS. Interestingly, the simple thiourea compound (**70**) was also found to be effective in inhibiting the function of MetRS. This result suggests that the structural modifications of two pharmacophoric features, the A-region and the R^2 fragment, may be significantly more effective in revealing new drug candidates than the B-region.

In order to investigate the detailed binding mode of the potent compound **27** (IC_{50} = 237 nM) as a MetRS inhibitor, it was docked into the ligand-binding site of *E. coli* MetRS.[19] The 3D-structure of compound **27** was built using the Sybyl molecular modeling program (Tripos, Inc.), and then the geometry was fully optimized using the Tripos force fields with the following non-default

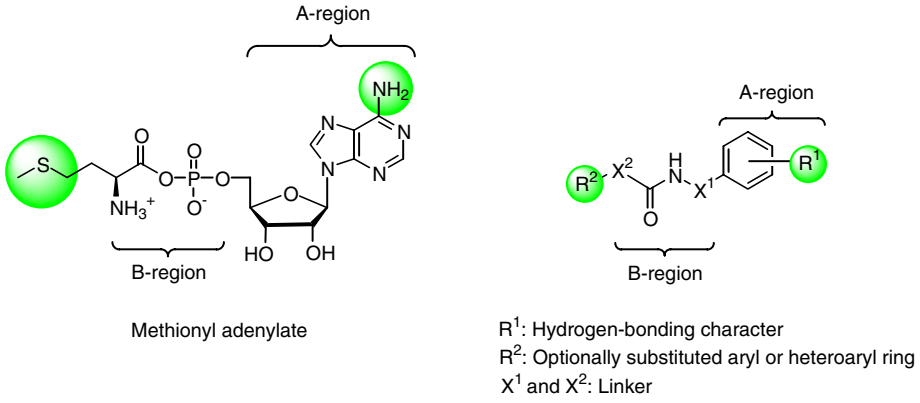
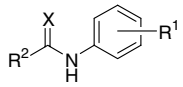


Figure 1. Pharmacophore query used in the 2D-database search.

Table 1. 91 Compounds generated by the virtual screening approach



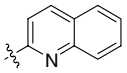
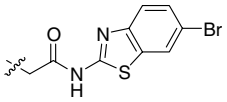
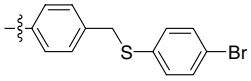
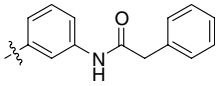
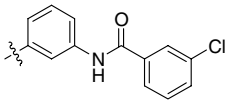
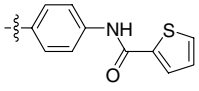
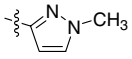
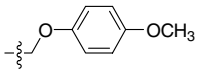
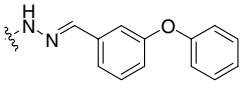
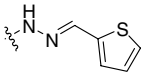
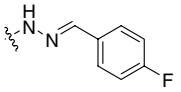
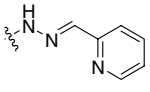
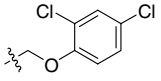
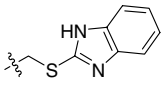
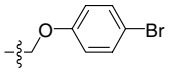
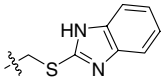
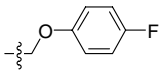
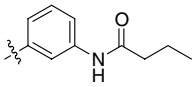
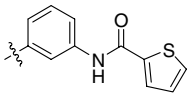
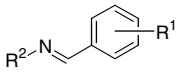
Compound	R^1	X	R^2
1	2-CO_2H	O	–$CH_2CONH(CH_2)_3CH_3$
2	2-CO_2H	O	–$CH_2CONHCH_2Ph$
3	4-CO_2H	O	
4	2-CO_2H	O	
5	4-CO_2H	O	
6	4-CO_2H	O	–CH_2COPh
7	3-CO_2H	O	
8	3-CO_2H	O	
9	3-CO_2H	O	
10	4-CO_2H	O	
11	4-CO_2H	O	
12	3-CO_2H	S	
13	3-CO_2H	S	
14	3-CO_2H	S	
15	3-CO_2H	S	
16	4-CO_2H	O	
17	4-NO_2	S	–$NHCH_2CH_2Ph$
18	3-CO_2H, 4-Cl	O	
19	4-$CONH_2$	O	

Table 1 (*continued*)

Compound	R^1	X	R^2
20	3-CO_2H	O	
21	3-CO_2H	O	
22	4-CO_2H	O	
23	4-CO_2H	O	



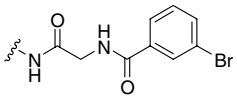
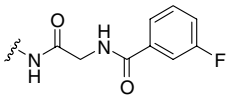
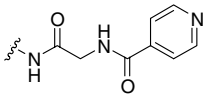
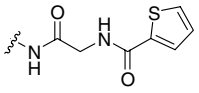
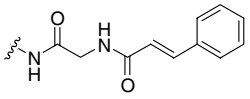
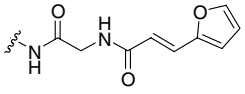
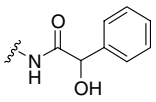
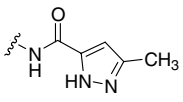
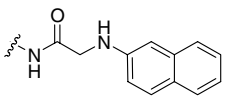
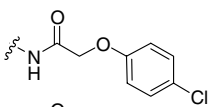
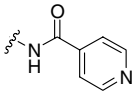
Compound	R^1	R^2
24	4-CO_2H	
25	4-CO_2H	
26	4-CO_2H	
27	4-CO_2H	
28	4-CO_2H	
29	4-CO_2H	
30	4-CO_2H	
31	4-CO_2H	
32	4-CO_2H	
33	4-CO_2H	
34	4-CO_2H	

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Table 1 (*continued*)

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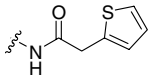
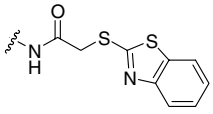
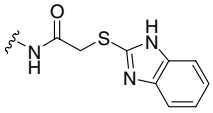
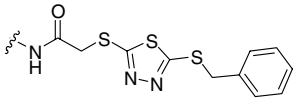
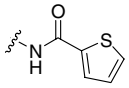
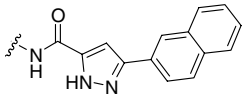
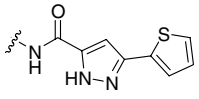
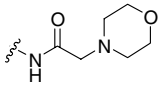
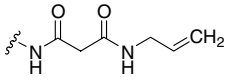
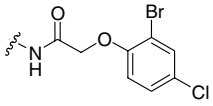
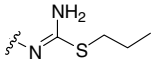
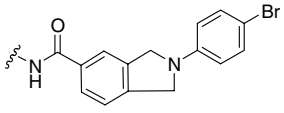
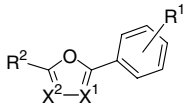
35	4-CO_2H	
36	4-CO_2H	
37	2-CO_2H	
38	4-CO_2H	
39	4-CO_2H	
40	4-CO_2H	
41	4-CO_2H	
42	4-CO_2H	
43	4-CO_2H	
44	4-CO_2H	
45	2-OH, 3-CO_2H	
46	4-CO_2H	



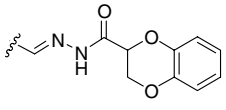
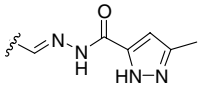
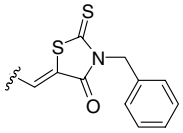
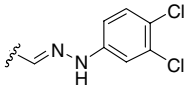
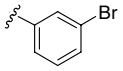
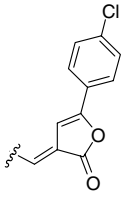
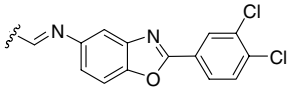
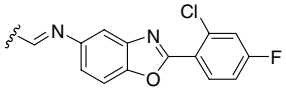
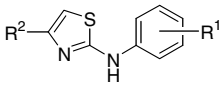
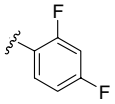
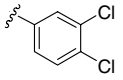
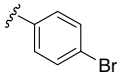
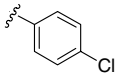
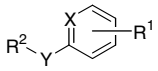
Compound	R^1	X^1	X^2	R^2
47	3-CO_2H	CH	CH	
48	3-CO_2H	CH	CH	
49	3-CO_2H	CH	CH	

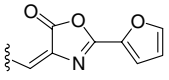
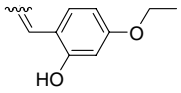
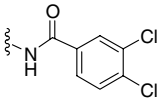
Table 1 (*continued*)

Compound	R^1	X^1	X^2	R^2
50	3-CO_2H, 4-Cl	CH	CH	
51	4-CO_2H	N	N	
52	3-CO_2H	CH	CH	
53	3-CO_2H	CH	CH	
54	3-CO_2H	CH	CH	



Compound	R^1	R^2
55	3-CO_2H	
56	3-CO_2H	
57	3-CO_2H	
58	3-CO_2H	
59	3-CO_2H	



Compound	R^1	X	Y	R^2
60	4-CO_2H	CH	NH	
61	4-CO_2H	CH	N	
62	4-CO_2H	CH	NH	

(*continued on next page*)

Table 1 (*continued*)

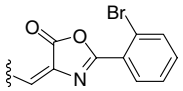
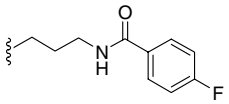
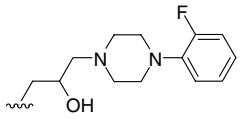
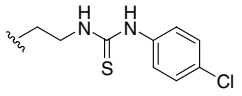
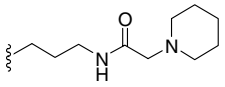
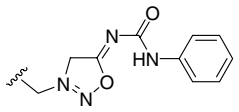
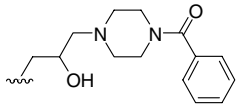
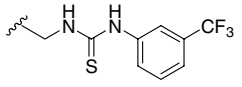
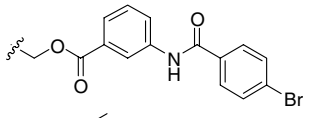
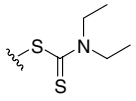
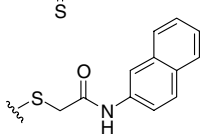
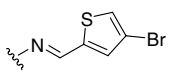
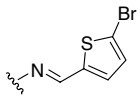
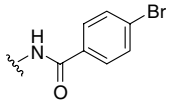
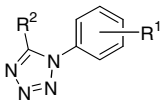
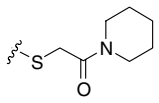
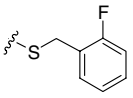
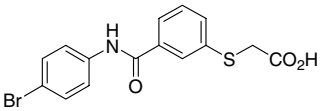
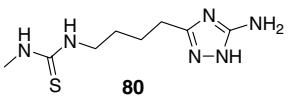
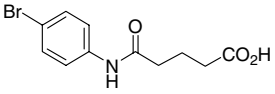
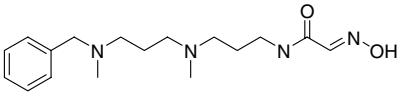
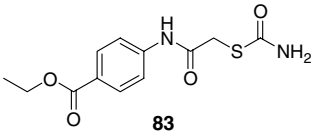
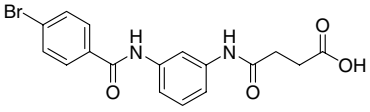
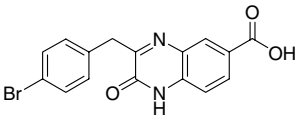
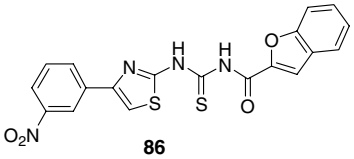
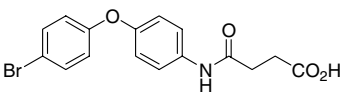
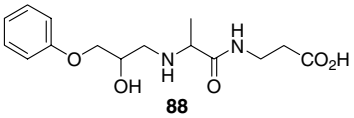
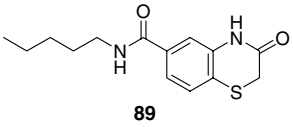
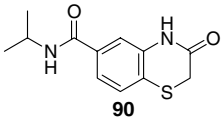
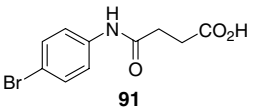
Compound	R^1	X	Y	R^2
63	4-CO_2H	CH	NH	
64	4-NO_2	CH	NH	
65	4-NO_2	CH	O	
66	4-NO_2	N	NH	
67	4-NO_2	CH	NH	
68	4-CO_2H	CH	CH_2	
69	4-NO_2	CH	O	
70	4-SO_2NH_2	CH	CH_2	
71	4-NO_2	CH	CO	
72	4-CO_2H	CH	CH_2	
73	4-CO_2H	CH	CH_2	
74	4-CO_2H	CH	NH	
75	3-CO_2H	CH	NH	
76	4-CO_2H	CH	CH_2	

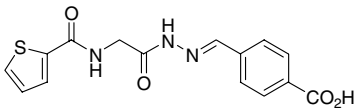
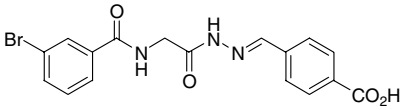
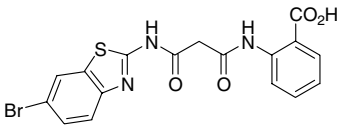
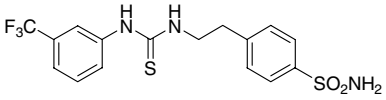
Table 1 (*continued*)



Compound	R^1	R^2
77	4-CO_2H	
78	4-CO_2H	
 **79**		 **80**
 **81**		 **82**
 **83**		 **84**
 **85**		 **86**
 **87**		 **88**
 **89**		 **90**
 **91**		

options (method: conjugate gradient, termination: gradient 0.001 kcal/mol Å, and max iterations: 10,000). The partial atomic charges were calculated by the Gasteiger–Hückel method in the Sybyl 6.9 program. Docking of the most potent compound (**27**) was carried out using the FlexiDock function of the Sybyl program. The active site of MetRS was defined as all of the amino acid residues within a radius of 4 Å from the bound methionyl adenylate (Met-$NHSO_2$-AMP). Hydrogen atoms were added to all of the residues of MetRS and water molecules were removed. To estimate the interaction energy between the ligand and the receptor-binding pocket, the Tripos force fields were used along with Gasteiger–Hückel and Kollman partial atomic charges for the ligand and enzyme, respectively. The initial location of compound **27** was pre-positioned using least-squares fitting on three pharmacophoric features: the N1 atom and N6 amine group of the adenine base

Table 2. Chemical structures and enzyme inhibitory activities of new MetRS inhibitors identified by virtual screening

Compound	Chemical structure	MW	IC_{50}[a] (μM)
27		331.25	0.237
24		404.21	22.8
4		434.26	30.5
70		403.44	47.7

[a] IC_{50} value against *E. coli* MetRS.

and the sulfur atom of the methionine side chain in methionyl adenylate were superimposed with the corresponding types of features (the carbonyl and hydroxyl group of the benzoic acid and the sulfur atom of thiophene) in compound **27**, respectively. The torsions of compound **27** were defined to fully consider the conformational flexibility, while the MetRS remained rigid. Compound **27** was docked into the binding pocket of MetRS with 20,000 genetic algorithm (GA) runs throughout the simulation. Based on the fitness score (energy), only the energetically favorable structures were analyzed and the lowest energy structure of compound **27** in the binding site of MetRS was selected for further refinement. Then, the obtained complex was fully optimized by energy minimization using Tripos force fields with minimization criteria (Powell method with a gradient of 0.01 kcal/mol Å). All computational work was done on a Silicon Graphics O2 R10000 workstation.

The docked model of compound **27** is shown in Figure 2. In the model, the carbonyl of the carboxylic acid group acts as a hydrogen bond acceptor for the main-chain N–H of Val326 (2.4 Å). The O–H of this group makes bifurcated hydrogen bonds with the carboxylate group of Asp296 (1.6 and 2.7 Å) and the amine group of Lys332 (1.9 and 2.1 Å). This result indicates that the carboxylic acid group at the aryl moiety plays an important role in the tight-binding with MetRS. The hydrophilic B-region (CH═N─NHCOCH$_2$NHCO), which was expected to act as a ribose-acylphosphate surrogate, engages in hydrogen bonds to the amide proton of Leu13 (1.7 Å) and the carboxylate oxygen atom of

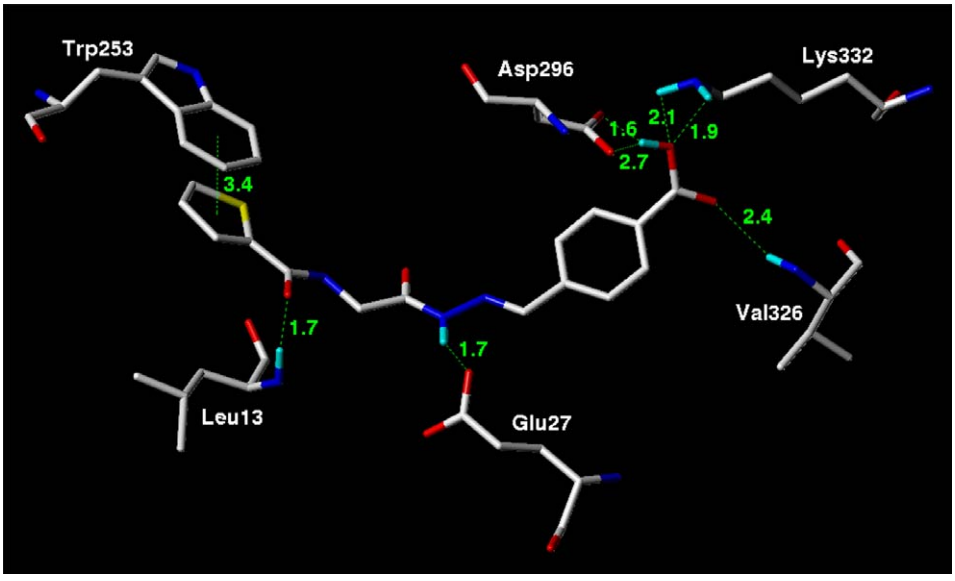


Figure 2. Proposed model of compound **27** bound to the *E. coli* MetRS binding site.

Glu27 (1.7 Å), respectively. In particular, the thiophene ring formed a parallel stack with the benzene ring of Trp253, with an interplanar stacking distance of 3.4 Å.

In summary, we have performed virtual screening of a chemical database of 508,143 commercially available chemicals to search for new methionyl-tRNA synthetase inhibitors. We were successfully identified four novel compounds for the inhibition of MetRS derived from *E. coli*, and particularly compound **27** showed the most potent inhibition, with an IC_{50} value of 237 nM against *E. coli* MetRS. The docking study of compound **27**, performed in the X-ray structure of the binding pocket of MetRS, revealed the important interactions with the enzyme. A synthetic SAR investigation with the leads is underway to find the optimal MetRS inhibitors.

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18. Macarron, R.; Mensah, L.; Cid, C.; Carranza, C.; Benson, N.; Pope, A. J.; Diez, E. *Anal. Biochem.* **2000**, *284*, 183; The assay mixture (20 mM HEPES, pH 7.5, 0.1 mM $EDTANa_2$, 150 mM NH_4Cl, 4 mM $MgCl_2$, 0.1 mg/ml BSA, 2 mM ATP, 0.4 mg/ml *E. coli* total tRNA, 5 μM methionine, and 0.25 μCi [^{35}S] methionine) was added to 96-well plate containing inhibitors. The reactions were initiated by the addition of the diluted MetRS enzyme. After a 10 min incubation at room temperature, the reaction was stopped by the addition of 150 μl of a continuously shaken suspension of YSi SPA beads (Amersham Pharmacia Biotech) at 1 mg/ml in 167 mM sodium citrate, pH 2. The beads were then allowed to settle for at least 45 min, and the plates were counted in a Microbeta (Wallac) liquid scintillator counter.
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